REMARKS

Reconsideration of this application is respectfully requested.

Claims 284-372 are pending in this application. Claims 284, 329, 331-335, 337, 348, 366-367 and 370-371 have been amended above by this Supplemental Amendment. Claims 373-375 have been added and no claims have been canceled. Accordingly, claims 284-375 are presented for further examination on the merits.

Before addressing the remaining issues in this application, the undersigned attorney would like to express his gratitude to the Examiner for the time and courtesy that was extended at the November 3, 1998 interview, also attended by Dean L. Engelhardt, Ph.D., Senior Vice President for the present assignee. It is believed that the frank discussions at the interview went a long way in clarifying the remaining issues in this application.

As a followup to the November 3rd interview, it is believed that this Second Supplemental Amendment will further clarify or narrow if not altogether resolve the issues that were raised in the January 6, 1998 Office Action, thereby placing this application in a better condition for allowance.

One prime example where the issues have been resolved involves the "self-signaling/self-indicating/self-detecting" terminology in claims 329 and 348. Claims 331 and 333 which depend from claim 329, and claims 370 and 371 which depend from claim 348, also contain the "self-signaling/self-indicating/self-detecting" terminology. In the amendments effected above, two of the terms, "self-signaling" and "self-detecting" have been deleted in all six claims in favor of "self-indicating." The latter term now describes the one or more modified nucleotides recited in those claims. This matter is discussed further below (see page 30, first full paragraph, through page 32).

Commensurate with their broad and complete disclosure, Applicants have also amended each of claims 284, 329, 331-333 and 337. In claim 284, the covalent attachment for the Markush nucleotide members (i, ii and iii) has been amended to recite that "such covalent attachment does not substantially interfere with double helix formation or nucleic acid hybridization." Support for the newly added phrase "or nucleic acid hybridization" is found in the specification as follows:

(i) nucleotide [Sig attached to base moiety BM]

page 93, first paragraph

> . . . The Sig moiety is covalently attached to the BM moiety of the nucleotide and when so attached is capable of signalling itself or makes itself self-detecting or its presence known and desirably or preferably permits the incorporation of the resulting nucleotide PM-SM-BM-Sig into or to form a double-stranded helical DNA or RNA or ***DNA-RNA hybrid*** and/or to be detectable thereon."
>
> [bold, italics & underline added]

(ii) nucleotide [Sig attached to sugar moiety SM]

page 94, first paragraph

> The Sig chemical moiety is covalently attached to the sugar SM moiety and said Sig chemical moiety when attached to said SM moiety is capable of signalling itself or making itself self-detecting or its presence known and preferably permits the incorporation of the ribonucleotide into its corresponding double-stranded RNA or a ***DNA-RNA hybrid***.
>
> [bold, italics & underline added]

(iii) nucleotide [Sig attached to phosphate moiety PM]

page 95, first paragraph

> . . . said Sig, when attached to said PM moiety being capable of signalling itself or making itself self-detecting or its presence known and desirably the nucleotide is capable of being incorporated into a double-stranded polynucleotide, such as DNA, RNA or ***DNA-RNA hybrid*** and when so incorporated therein is still self-detecting.
>
> [bold, italics & underline added]

Other support for the foregoing amendments to claim 284 is found on page 96, first paragraph, where it is disclosed:

> . . . The chemical moiety Sig so attached to the nucleotide PM-SM-BM is capable of rendering or making the resulting nucleotide, now comprising PM-SM-BM with the Sig moiety being attached to one or more of the other moieties, self-detecting or signalling itself or capable of making its presence known per se, when incorporated into a polynucleotide, such as ***double-stranded DNA, a double-stranded RNA or a double-stranded DNA-RNA hybrid***. ***The Sig moiety desirably should not interfere with the capability of the nucleotide to form a double-stranded polynucleotide containing the special Sig-containing nucleotide in accordance with this invention and, when so incorporated therein, the Sig-containing nucleotide is capable of detection, localization or observation***.
>
> [bold, italics & underline added]

In claim 329 which is directed to a process for determining the sequence of a nucleic acid of interest, rather minor amendments have been effected to the first and third steps recited in the claim language. Three changes have been made to the first incorporating step of claim 329. As now amended, the first step recites "incorporating one or more modified nucleotides or an oligo- or polynucleotide comprising one or more modified nucleotides into a nucleic acid or nucleic acid fragments complementary to said nucleic acid of interest . . ." Second, as discussed above, the terms "self-signalling" and "self-detecting" have been deleted in favor of "self-indicating." This latter change also affects the third step of claim 329. Third, the phrase -- or a portion thereof -- has been added to the end of the first step, particularly since a fragment or fragments can only be complementary, by definition, to a portion of a nucleic acid of sequence. In the third step of detecting, three changes have also been made (four, if the "self-signalling/self-indicating" deletion is counted). First, the phrase "specific segment of" has been deleted. Second, the word "fragment" has been pluralized to -- fragments -- . Third, the phrase -- or nucleotides -- has been added at the end of the claim. The latter two changes are believed to merely conform the elements in the third step with their antecedent basis. That is to say, the first step recites "one or more modified nucleotides" and "fragments" and both recitations have now been carried over directly to the third step by means of the amendments to claim 329.

Claims 331 and 337 are directed to sequencing and preparation processes, respectively. Both claims have been amended by three deletions to the Markush nucleotide members (i, ii and iii). In each of these members, the phrase "and such covalent attachment does not substantially interfere with double helix formation." Because double helical formation is neither an objective of nor a concern in sequencing or preparing the modified nucleotides of the present invention, it is believed that the language of claims 331 and 337 is improved by deleting a characteristic that is not necessary to carry out the sequencing or preparation process.

Again, in an effort to define their invention more clearly, Applicants have amended claims 366-367 by replacing the word "external" with a clearer term -- terminal -- to describe the modified nucleotide. It is believed that both claims have been rendered clearer and more definite by the foregoing change.

New claims 373-375 have been added above. Claim 373 is in large part based upon claim 329 with the exception that claim 373 comprises four steps in the sequencing process, versus the three steps in claim 329. Essentially, the addtional step in claim 373 is directed to the introduction of the labeled nucleic acid or nucleic acid fragments into a sequencing gel (step 2). In line with the language of new claim 373, claims 332-335, all of which depend from claim 329, have also been amended to depend from new claim 373. New claims 374 and 375 depend from claims 373 and 374, respectively. Both claims recite "wherein said detecting step comprises localizing said labeled nucleic acid or said labeled nucleic acid fragments by means of said self-indicating nucleotide or nucleotides." Support for the "localizing" language in new claims 374 and 375 is taken from the specification, page 96, first paragraph (" . . . the Sig-containing nucleotide is capable of detection, localization or observation"), discussed and quoted *supra* (see the previous page of this Amendment, page 17, first full paragraph).

Applicants sincerely believe that the foregoing amendments and new claims 373-375 do not constitute the insertion of new matter into their disclosure. Entry of the above amendments is respectfully requested.

During the November 3rd interview, and as indicated in the Interview Summary dated the same day, the Examiner indicated that new matter issues regarding template dependent or template independent synthesis, 3' end labeling and sequencing practice were withdrawn. The Examiner also indicated that Applicants' response was expected shortly to clear up the remaining issues. As discussed during the interview, the following are believed to be the remaining issues under the rejection for new matter:

Subject Matter/Recitation	Affected Claims
Linkage Group	
"said linkage group contains an amine"	339, 350, 353, 356
"said amine comprises a primary amine"	340, 351, 354, 357
"said linkage group does not substantially interfere with formation of the signaling moiety or detection of the detectable signal"	341, 358
5' End Labeling	
"wherein the labeled oligo- or polynucleotide of interest prepared by said incorporating step comprises at least one external modified nucleotide"	366
Electrophoretic Separation Step	
"wherein said separating step is carried out electrophoretically"	368
Monosaccharide	
"SM is a monosaccharide"	284, 331, 337, 348

Self-Signalling/Self-Indicating/Self-Detecting

"self-signalling or self-indicating or self detecting" 329, 348, 370, 371

The remarks that follow are directed to the five items listed above.

1. ***Linkage Group***

With respect to the linkage group defined by claims 339-341, 350-351, 353-354, 356-357 and 358, all of which are dependent, it is believed that a substantial if not thorough discussion was previously given in Applicants' July 24, 1998 Supplemental Response to Applicants' July 6, 1998 Amendment Under 37 C.F.R. §1.116. For the sake of completeness and the Examiner's convenience, the following chart was provided in support of the language in the claims at hand.

Claims	Recitation	Support in Specification
339, 350 353, 356	**wherein said linkage group contains an amine**	**Page 11, 2nd full ¶** ("carbon-nitrogen bonds") **Page 11, 3rd full ¶** ("It is even more preferred that the chemical linkage group be derived from a primary amine, and have the structure $-CH_2-NH-$, since such linkages are easily formed utilizing any of the well known amine modification reactions. Examples of preferred linkages derived from allylamine and allyl-(3-amino-2-hydroxy-1-propyl) ether groups . . . Although these linkages are preferred, others can be used, including particularly olefinic linkage arms with other modifiable functionalities such as thiol, carboxylic acid, and expoxide functionalities.) **Page 98, 1st ¶** ("Accordingly, the Sig component or chemical moiety of nucleotides of this invention can be directly covalently attached . . . via a chemical linkage or linkage arm as described in U.S. patent application Ser. No. 225,223, . . . The various linkages identifed in U.S. Ser. No. 225,223 are applicable to and useful in the preparation of the special nucleotides of this invention.") **Originally filed claims 79-80, 90, 202-203**
340, 351	**wherein said amine comprises**	**Page 11, 3rd full ¶**

354, 357	**a primary amine**	**Page 13** ("to employ olefins with primary amine functional groups, such as allylamine (AA) or allyl-(-3-amino-2-hydroxy-1-propyl) ether (NAGE)") Page 16, schema ("allylamine") Page 18, 3rd ¶ ("Examples include .") Page 98, 1st ¶, *supra*.
341, 358	**wherein said linkage group does not substantially interfere with formation of the signalling moiety or detection of the detectable signal**	**Page 7, 2nd ¶** (Fourth, the detection system should be capable of interacting with probe substituents incorporated into both single-stranded and double-stranded polynucleotides in order to be compatible with nucleic acid hybridization methodologies. To satisfy this criterion, it is preferable that the probe moiety be attached to the purine or pyrimidine through a chemical linkage or "linker arm" so that it can readily interact with antibodies, other detector proteins, or chemical reagents.") **Page 11, 2nd ¶** ("The linkage or group joining moiety A to base B may include any of the well known bonds including carbon-carbon single bonds, or carbon-oxygen single bonds. However, it is generally preferred that the chemical linkage include an olefinic bond at the ∂-position relative to B. The presence of such an ∂-olefinic bond serves to hold the moiety A away from the base when the base is paired with another in the well known double-helix configuration. This permits interaction with polypeptide to occur more readily, thereby facilitating complex formation. Moreover, single bonds with greater rotational freedom may not always hold the moiety sufficiently apart from the helix to permit recognition by and complex formation with polypeptide.) **Page 96, 1st ¶** ("By way of summary. . . . The chemical moiety Sig so attached to the nucleotide P-S-B is capable of rendering or making the resulting nucleotide, now comprising P-S-B with the Sig moiety being attached to one or more of the other moieties, self-detecting or signalling itself or capable of making its presence known per se, when incorporated into a polynucleotide, especially a double-stranded polynucleotide. . . The Sig moiety desirably should not interfere with the capability of the nucleotide to form a double-stranded polynucleotide containing the special Sig-containing nucleotide . . . and, when so incorporated therein, the Sig-containing nucleotide is capable of detection, localization or observation.) **Page 97, 2nd ¶** (" . . . the Sig component could comprise any chemical moiety which is attachable either directly or through a chemical linkage or linker arm to the

> nucleotide, such as to the base B component therein, or the sugar S component therein, or the phosphoric acid P component thereof.)

The above information is found on pages 16-17 of Applicants' July 24, 1998 Supplemental Response, as well as on pages 10-12 of the Declaration of Dr. James J. Donegan In Support of Adequate Description, the latter attached to the former response as Exhibit A. As indicated in his Declaration (Paragraph 14), it is Dr. Donegan's opinion and conclusion that the specification reasonably conveys that the inventors were in possession of the specific linkage group subject matter of claims 339-341, 350-351, 353-354 and 356-358 at the time of their 1982 application filing.

In reviewing the above chart and claims 339, 350, 353 and 356 in particular, Applicants wish to point out that the disclosure is broader than the claim language directed to "said linkage group contains an amine." For example, it is disclosed in the specification (page 11, second full paragraph) that:

> The linkage or group joining moiety A to base B may include any of the well known bonds including carbon-carbon single bonds, carbon-carbon double bonds, **carbon-nitrogen single bonds**, . . .
>
> [bold added]

A carbon-nitrogen single bond clearly encompasses an amine, including a primary amine, the latter disclosed in the third paragraph on page 11:

> It is even more preferred that the chemical linkage group be derived from a **primary amine**, and have the structure $-CH_2-NH-$, since such linkages are easily formed utilizing any of the well known amine modification reactions. Examples of preferred linkages derived from allylamine and allyl-(3-amino-2-hydroxy-1-propyl) ether groups . . . Although these linkages are preferred, others can be used, including particularly olefinic linkage arms with other modifiable functionalities such as thiol, carboxylic acid, and expoxide functionalities.
>
> [bold added]

The term "primary amine," cited in the above passage, is, of course, the subject matter of claims 340, 351, 354 and 357.

With respect to the other claims at issue, 341 and 358, both recite that "said linkage group does not substantially interfere with formation of the signaling moiety or detection of the detectable signal." The passages from the specification quoted in the chart above clearly establish that the probe moiety:

- [should] be attached to the purine or pyrimidine through a chemical linkage or "linker arm" so that it can readily interact with antibodies, other detector proteins, or chemical reagents.

And that the chemical linkage:

- serves to hold the moiety A away from the base when the base is paired with another in the well known double-helix configuration

It is believed, therefore, that the specification supports the language of claims 341 and 358.

2. ***5' End Labeling***

Regarding the issue of labeling the 5' end of an oligo- or polynucleotide, it is believed that the specification supports and enables this embodiment as may be covered by claim 366 (and claim 367). These claims depend from the process claim of 348 directed to detecting the presence of an oligo- or polynucleotide of interest in a sequencing gel. Step B in claim 348 calls for "incorporating said one or more chemically modified nucleotides into said oligo- or polynucleotide, thereby preparing a labeled oligo- or polynucleotide of interest, said labeled oligo- or polynucleotide of interest comprising one or more chemically modified nucleotides . . ." As amended above, claim 366 now recites "wherein the labeled oligo- or polynucleotide of interest prepared by said incorporating step comprises at least one terminal modified nucleotide." Claim 367 also recites the same "terminal" language.

It is believed that the specification supports and enables 5' end labeling as covered by claim 366 (and 367). As an illustration, Example V on page 57 provides a means for 5' end labeling through the oxygen or phosphorus of the phosphate moiety of a nucleotide, including the 5' phosphate in an oligo- or polynucleotide using for example the procedure of Halloran and Parker, J. Immunol. 96:373 (1966)[1]. As another illustration, RNA ligase disclosed in the specification (page 20, first paragraph) also discloses 5' end labeling through phosphate labeling, that is, by attaching Sig to the 5' phosphate.

Applicants also believe that at the time their application was originally filed in 1982, a person skilled in the art could have effected the 5' end labeling of an oligo- or polynucleotide with at least one external modified nucleotide, as set forth in claims 366 and 367. Using conventional enzymes

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[1] **Applicants wish to eagerly point out that in Example V, the cited 1966 Halloran and Parker article actually discloses in Figure 1 on page 374, two separate reaction procedures for modifying the 5' phosphate of a nucleotide (either DNA or RNA). The first reaction procedure, designated Reaction 1 in Figure 1, involves formation of a phosphodiester bond with protein seryl and threonyl residues. The second reaction procedure, designated Reaction 2, involves N-P bond formation with protein epsilon-amino groups. Figure 1 from Halloran and Parker is reproduced below:**

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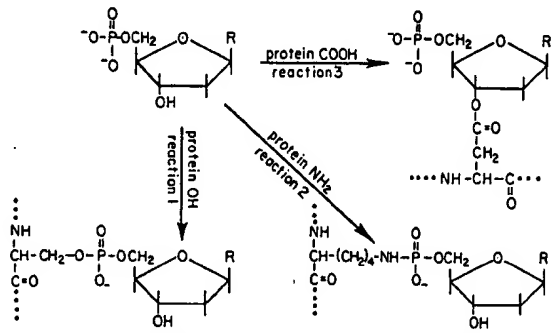


Figure 1. Possible reactions of nucleotides with proteins in the presence of carbodiimides. Very unstable products such as acyl phosphates are not shown.

and techniques conventionally known in the early 1980s, such as nucleases, e.g. DNases, phosphatases, kinases and RNA ligase, the skilled artisan could have carried out 5' end labeling in accordance with Applicants' claimed invention as defined in claim 366.

3. ***Electrophoretic Separation Step***

[4] Concerning the electrophoretic separating step in claim 368, it is believed that a thorough discussion of this issue was presented in Applicants' July 24, 1998 Supplemental Response that included Dr. Donegan's Declaration. That discussion in their Supplemental Response began on page 25, second full paragraph, and continued through the first line on page 29. For the sake of completeness, that discussion is reiterated below:[2]

. . . it is Dr. Donegan's opinion and conclusion that the specification fully supports the subject matter of this step and claim. According to Dr. Donegan in his Declaration, at the time this application was filed in 1982, electrophoretic separation was an intrinsic step in sequencing gel practice. In several of the publications and definitions discussed above, electrophoretic separation or similar terminology is used to describe sequencing gels and sequencing gel practice. Dr. Donegan offers the following publications and definitions as support for his position:

Maxam and Gilbert's 1977 PNAS paper:

> We have developed a new technique for sequencing DNA molecules. The procedure determines the nucleotide sequence of a terminally labeled DNA molecule by breaking it at adenine, guanine, cytosine, or thymine with chemical agents. Partial cleavage of each base produces a nested set of radioactive fragments extending from the labeled end to each of the positions of that base. Polyacrylamide gel **electrophoresis** resolves these single-stranded fragments, their sizes

[2] **While this discussion quotes a number of publications and other materials (eleven in all) which were cited in their July 24, 1998 Supplemental Response and provided as exhibits to Dr. Donegan's Declaration, Applicants have not provided any copies herewith.**

reveal *in order* the points of breakage. The autoradiograph of a gel produced from four different chemical cleavages, each specific for a base in a sense we will describe, then shows a pattern of bands from which the sequence can be read directly. This method is limited only by the resolving power of the polyacrylamide gel. . .
Maxam and Gilbert (1977), page 560, left column, 1st ¶, [bold added]

Sanger's 1977 PNAS paper:

Principle of the Method. Atkinson *et al.* (4) showed that the inhibitory activity of 2′,3′-dideoxythymidine triphosphate (ddTTP) on DNA polymerase I depends on its being incorporated into the growing oligonucleotide chain in the place of thymidylic acid (dT). Because the ddT contains no 3′-hydroxyl group, the chain cannot be extended further, so that termination occurs specifically at positions where dT should be incorporated. If a primer and template are incubated with DNA polymerase in the presence of a mixture of ddTTP and dTTP, as well as the other three deoxyribonucleoside triphosphates (one of which is labeled with ^{32}P), a mixture of fragments all having the same 5′ and with ddT residues at the 3′ ends is obtained. When this mixture is fractionated by **electrophoresis** on denaturing acrylamide gels the pattern of bands shows the distribution of dTs in the newly synthesized DNA. By using analogous terminators for the other nucleotides in separate incubations and running the samples in parallel on the gel, a pattern of bands is obtained from which the sequence can be read off as in the other rapid techniques mentioned above.
Sanger et al. (1977), page 5463, left column, penultimate ¶, [bold added]

Barnes' 1978 JMB paper:

. . . A key observation made by Sanger and his colleagues was that **electrophoresis on acrylamide gels** can resolve DNA molecules differing in length by a single nucleotide over the range of 20 to 140 nucleotides, if the molecules to be analysed all have the same sequence and share the same 5′ end (Barrell *et al.* 1976). Maxam and Gilbert (1977) use **similar high-resolution acrylamide gels** in a DNA sequencing method that uses the principle of end-labelling and base-specific partial chemical cleavage. . .
Barnes (1978), page 83, [bold added]

Winter and Brownlee (1978):

(3) **Sequencing gel**. The conditions are essentially as described in Simonosits et al. [1] but were **adapted for use with thin gels** [18]. 1 μl aliquots of labelled tRNA were dried and digested in 2 μl as

follows: 0.003 units RNase T_1, 0.1 M Tris-HCl, 10 mM EDTA pH 7.5, 0°C, 2 min and 10 min; 2 pg RNase A, pH 7.5 buffer (as for T_1 RNase), 0°C, 5 min and 30 min; 0.4 units RNase U_2, 8.75 M urea, 20 mM sodium acetate, 2 mM EDTA, 50°C, 5 min; 0.0014 units RNase Phyl, 10 mM sodium acetate, 1 mM EDTA, pH 5.9, room temperature, 1½ min and 20 min; formamide containing 1 µl 1 M magnesium acetate per ml, 100°C, 30 min. For the ladder, traces of magnesium ion were added to the hot formamide to catalyse the degradation (R.H. Symons and G.P. Winter, unpublished). Partial alkali cleavage could have been used for the ladder since the mixture of cyclic phosphate and 2' and 3' phosphates so generated are not attached to the radioactively labelled fragments. With 5' labelled sequences, however, the different mobilities of small oligonucleotides with cyclic and open phosphates introduces extra bands into the early portions of the ladder [1]. Time points were combined before running and a control of undigested $tRNA^{Phe}$ was included. Reactions were stopped by adding 2 µl of formamide dye mixture and heating at 100°C for 1 min. 2 µl of **sample were applied to the thin gel which was electrophoresed** at 1.6 kV for 4 h; the remaining 2 µl was applied after reheating and the **gel electrophoresed** for a further 2 h. The gel was exposed to preflashed film for five days [20]. [bold added]

Maxam and Gilbert's 1980 Methods in Enzymology techniques paper:

Gel sequencing methods have traditionally employed versions of a pH 8.3 polyacrylamide gel described by Peacock and Dingman[62] and adapted for small single-stranded DNA molecules by Maniatis *et al.*[63]
Page 540, first full paragraph

Garoff and Ansorge (1981):

The new and rapid DNA sequencing techniques involve the generation of a set of oligonucleotides, which have one end in common and the other end varying in length with a single nucleotide, and the subsequent separation of the oligonucleotides on denaturing polyacrylamide gels. page 430, right column, 1st ¶

A Dictionary of Genetic Engineering, Oliver and Ward, page 100:

> **sequencing gel** A long polyacrylamide slab gel which has sufficient resolving power to separate single-stranded fragments of DNA or RNA which differ in length by only a single nucleotide. **Electrophoresis** is carried out at high voltage and with the gel in a vertical position. Urea is usually included in the gel mixture as a denaturing agent. This prevents internal base pairing within the

> single-stranded molecules and ensures that their relative speed of migration is solely dependent on their length. Such gels are used to separate the radioactively labelled products of, for example, the Maxam-Gilbert or the Sanger sequencing reactions. [bold added]

Dictionary of Biochemistry and Molecular Biology, Stenesh, page 437:

> **sequencing gel** A long, thin polyacrylamide gel slab used for nucleic acid sequencing.

Oxford Dictionary of Biochemistry and Molecular Biology, Smith et al., editors, page 594:

> **sequencing gel** a polyacrylamide gel run to resolve oligonucleotides produced in a DNA sequencing procedure. *See* **chain-termination method, chemical cleavage method.** [bold & italic in original]

Dictionary of Plant Genetics and Molecular Biology, G. Miglani, page 258:

> **sequencing gel:** A long, polyacrylamide salt gel that has sufficient resolving power to separate single-stranded fragments of DNA or RNA which differ in length by only a single nucleotide. **Electrophoresis** is carried out at high voltage and with the gel in a vertical position. [bold added]

Hindley's book of DNA Sequencing, "Preliminary Remarks" in the Introduction (Chapter 1):

> 1.1 Preliminary Remarks
> The present art of DNA sequencing has its origins in a variety of different fields of nucleic acid enzymology and chemistry. Indeed as early as 1970 our knowledge and understanding of these fields was, in theory, sufficiently far advanced to anticipate the development of the modern rapid methods but two obstacles had first to be overcome to convert these ideas into reality. The first was the problem of separating the oligonucleotides, generated in the sequencing reactions, in a rapid convenient and reproducible manner and displaying them as an ordered set of fragments according to their chain length. While the technique of homochromatography, in which a random mixture of polynucleotides of all possible chain lengths is used to develop a chromatogram (Brownlee and Sanger, 1969), was an important step in this direction, it was through the development of gel electrophoretic techniques that this problem was finally solved. **All the methods to be described rely on the extraordinary resolving**

> **power of polyacrylamide gels** run under denaturing conditions to achieve the final separations; much effort has gone into perfecting such systems so as to optimise their resolving properties. . ."
> [Hindley, DNA Sequencing, page 1, bold added]

Thus, according to Dr. Donegan and based upon the documents referenced in Subparagraphs 16A-16H of his Declaration, and further based upon his own experience and knowledge at the time this application was filed in 1982, it would have been understood that the practice and use of sequencing gels intrinsically involved electrophoretic separation as set forth in claim 368.

4. ***Monosaccharide***

All of the present claims are directed to the use of nucleotides in which "SM is a monosaccharide." It is believed that the term "monosaccharide" meets both the written description and enablement strictures for patentability under 35 U.S.C. §112, first paragraph. To begin with, the specification discloses at several places this term with the structural definition for the special nucleotides of Applicants' claimed invention. For example, on page 90, last paragraph, it is discloses:

> The special nucleotides of this invention include a phosphoric acid P moiety, a sugar or ***monosaccharide SM moiety***, a base BM moiety, a purine or a pyrimidine and a signalling chemical moiety Sig covalently attached thereto, either to the PM, SM or BM moiety. . .
> [bold, italics & underline added]

Later, on page 93, first paragraph, it is further disclosed:

> The special nucleotides in accordance with this invention, as indicated hereinabove, in addition to the PM, SM and/or BM moieties. Of special interest in accordance with the practices of this invention would be those nucleotides having the general formula,
> PM – SM –BM – Sig
> wherein PM is the phosphate moiety including mono-, di-, tri-, or tetraphosphate, SM the sugar or ***monosaccharide moiety***, BM the base moiety, . . . [bold, italics & underline added]

Again, on page 103, second paragraph, the following is disclosed:

> The special nucleotides of this invention and polynucleotides including such nucleotides, either single-stranded or double-stranded polynucleotides, DNA and/or RNA, comprising the components, phosphate moiety PM, the sugar or ***monosaccharide moiety SM***, the base moiety BM, a purine or a pyrimidine, and the signalling or self-detecting moiety, Sig, covalently attached to either the PM, SM or BM moieties, as indicated hereinabove, have many uses and utilities. . .
> [bold, italics & underline added]

Just as significantly, the original claims contain the same reference to "S" [SM] as being a sugar or monosaccharide moiety. Original claims 1, 142 and 143 recite the term "monosaccharide moiety" in referring to the component S of the special nucleotide:

> 1. A nucleotide having the general formula P–S–B–Sig wherein P is the phosphoric acid moiety, S the sugar or ***monosaccharide moiety***, . . . [bold, italics & underline added]

> 142. A nucleotide having the general formula
> P – S – B – Sig
> wherein P is the phosphoric acid moiety, S the sugar and ***monosaccharide moiety***, . . . [bold, italics & underline added]

> 143. A nucleotide having the general formula P–S–B, wherein P is the phosphoric acid moiety, S the sugar or ***monosaccharide moiety*** . .
> [bold, italics & underline added]

Thus, from a written description standpoint, the term "monosaccharide" in the instant claims is well-based and clearly supported.

In terms of enablement, practicing the present invention directed to SM being a monosaccharide moiety was well within the ambit of the skilled artisan, who could have readily utilized monosaccharides for the purposes described in the specification without undue experimentation. It was and continues to be well-recognized in the art that monosaccharides are simple sugars defined as aldehydes or ketones with multiple hydroxyl groups. In other words, monosaccharides are polyhydroxy alcohols containing an aldehyde or a ketone group. The following scientific and technical

definitions for "monosaccharides" are offered in further support of this definition.

Stenesh, Dictionary of Biochemistry and Molecular Biology, 2nd Edition, John Wiley & Sons, New York, 1989, page 309

> **monosaccharide** A polyhydroxy alcohol containing either an aldehyde or a ketone group: a simple sugar. [bold in original]

Stryer, Biochemistry, 3rd Edition, W. H. Freeman And Company, New York, 1988, page 332

> **MONOSACCHARIDES ARE ALEDHYDES OR KETONES WITH MULTIPLE HYDROXYL GROUPS**
> Monosaccharides, the simplest carbohydrates, are aldehydes or ketones that have two or more hydroxyl groups, . . . [bold in original]

Without any undue experimentation, a person skilled in the art could have practiced Applicants' claimed invention within the context of SM being a monosaccharide moiety, particularly because sugar chemistry applicable to monosaccharides was generally known in the art and available at the time of the present invention. Furthermore, Applicants disclose and exemplify in their specification signficant sugar chemistry, including Example V (page 57 in the specification), discussed *supra* (see this Amendment, page 24, including footnote 1).

5. ***Self-Signalling/Self-Indicating/Self-Detecting***

As indicated in their opening remarks, Applicants have amended the language in each of claims 329, 331, 333, 348, 370 and 371 by expunging the terms "self-signaling" and "self-detecting," thereby leaving "self-indicating" as a characteristic of the one or more modified nucleotides recited in those claims. As also indicated above, it is believed that the term "self-indicating" is synonymous with and equivalent to the other two terms, "self-signaling" and "self-detecting." Further, although it is permissible under U.S. patent practice to claim elements in the alternative which are

functional equivalents for each other, Applicants are willing to streamline the claim language in accordance with the Examiner's position.

Before leaving the subject of clarity with respect to the term "self-indicating," Applicants wish to point out that in their July 24, 1998 Supplemental Response that two U.S. patents were offered as evidence where such language had been recited in issued U.S. claims. Those two documents were U.S. Patent Nos. 4,649,121 and 5,233,044, copies of which were attached to Applicants' July 24th Supplemental Response as Exhibits C and D, respectively Attention was directed to claims 6 and 7 in the '121 Patent, and claim 1 in the '044 Patent.

In addition to the two aforementioned patents, Applicants have uncovered two other U.S. patents that recite the language "self-indicating" in the issued claims. Information for the two U.S. patents is listed below:

U.S. Patent No.	Claim Number(s)	Recitation	Exhibit No.
4,981,653	1-8	self-indicating assay device	1
	9	self-indicating reagent strip	
4,408,202	1, 20, 50	self-indicating reagents	2

Other scientific publications also recognize and use the terminology "self-indicating" with respect to substrates. In this regard, Applicants can point to the following four articles:

> Atherton et al., "***Self-indicating*** Activated Esters for Use in Solid Phase Peptide Synthesis. Fluorenylmethoxycarbonylamino Acid Derivatives of 3-Hydroxy-4-oxodihydrobenzotriazine," Journal Chemical Society, Chemical Communications 0(24):1763-1765 (1986) [copy attached as Exhibit 3] [bold & italic added]

> Valcour et al., "Evaluation of a Kinetic Method for Prostatic Acid Phosphatase with Use of ***Self-Indicating*** Substrate, 2,6-Dichloro-4-Nitrophenyl Phosphate," Clinical Chemistry 35(6):939-945 (1989) [Exhibit 4] [bold & italic added]

Rocco, R. M., "Fluorometric Determination of Alkaline Phosphatase in Fluid Dairy Products: Collaborative Study," J. Assoc. Off. Anal. Chem. 73(6):542-549 (1990) [Exhibit 5]

> The purpose of the present study was to collaboratively examine a new fluorometric assay for ALP in dairy products (3). The method is based on a fluorometric substrate called *Fluorophos*®, which, when acted upon by ALP, is converted to a highly fluorescent product. This fluorometric quantitative assay is the first dairy product ALP test that permits continuous and direct measurement of the released reaction product from a ***self-indicating*** substrate. . .
>
> [page 542, right column, first full ¶, bold, italic & underline added]

Osawa et al., "Prostatic Acid Phosphatase Assay with ***Self-Indicating*** Substrate 2,6-Dichloro-4-acetylphenyl Phosphate," Clinical Chemistry 41(2):200-203 (1995) [Exhibit 6] [bold & italic added]

> We characterize six ***self-indicating*** substrates . . .
>
> [page 200, abstract; bold, italic & underline added]

> **Discussion**
>
> To overcome several disadvantages involved in conventional methods for PAP activity (1-8), we have developed a new assay and described its performance. DCAPP, a ***self-indicating*** synthetic substrate, has played a key role.
>
> [page 202, right column, first ¶; bold, italic & underline added]

> In summary, for the measurement of PAP activity, our kinetic method involving the ***self-indicating*** substrate DCAPP showed satisfactory performance on automated analyzers. . .
>
> [page 203, left column, last ¶; bold, italic & underline added]

Copies of the four articles cited above have been attached as Exhibits 3-6, as indicated above.

In light of the above amendments to the claims, the submitted exhibits and foregoing remarks, it is believed that all outstanding issues affecting this application and the present claims have been addressed. An indication of allowability for claims 284-375 is respectfully requested.

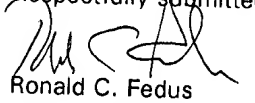
SUMMARY AND CONCLUSIONS

Claims 284-375 are presented for further examination on the merits. Claims 284, 329, 331-335, 337, 348, 366-367 and 370-371 have been amended. Claims 373-375 have been added. No other claims have been amended, canceled or added by this Amendment.

No extension fee is deemed necessary in connection with the filing of this Second Supplemental Amendment, fees having been previously authorized in Applicants' July 6, 1998 Amendment Under 37 C.F.R. §1.116, both for Applicants' request for the withdrawal of the finality of the January 6, 1998 Office Action and their three month extension request. As set forth in the attached transmittal, authorization is given for the additional claim fee of $204.00. This additional $204.00 claim fee results from the submission of new claims 373-375 (1 independent claim, 2 dependent claims) and the extra claims presented by way of multiple dependency added to claims 331, 332, 333 and 335 (4 additional claims). Thus, seven claims and one independent claim have been added and paid for herein. It is believed that a fee for multiple dependency was previously paid. No other fee or fees are believed due. If, however, any other fee or fees are deemed necessary in connection with this Second Supplemental Amendment, authorization is hereby given to charge the amount of any such fee to Deposit Account No. 05-1135.

If a telephone conversation would further the prosecution of the present application, Applicants' undersigned attorney request that he be contacted at the number provided below.

Respectfully submitted,



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